OBSERVATIONS: BRIEF RESEARCH REPORTS

Implementation of a SARS-CoV-2 Genotyping Panel for Prompt Omicron Variant Identification: A Pragmatic Tool for Clinical Laboratories

Background: The emergence of the SARS-CoV-2 Omicron variant has important clinical and therapeutic implications. Certain SARS-CoV-2 monoclonal antibody therapies are ineffective or have reduced efficacy against this variant (1). Whole-genome sequencing (WGS) is the gold standard for variant identification, but it requires costly equipment and can be labor intensive, and the results are generally not available to inform real-time therapeutic decisions. An assay that quickly differentiates Omicron from other circulating SARS-CoV-2 strains may help with clinical decision making.

Objective: To determine if a real-time nucleic acid amplification–based SARS-CoV-2 mutational panel can accurately identify SARS-CoV-2 variants, including Omicron.

Methods and Findings: Many Omicron sequences include the ΔH69/V70 deletion mutation, resulting in the inability of certain SARS-CoV-2 nucleic acid amplification tests (NAATs) to detect the spike gene target (S gene target failure [SGTF]), while still detecting SARS-CoV-2 RNA (2, 3). With the initial reports of Omicron, we screened SARS-CoV-2 NAAT–positive specimens tested within the Mass General Brigham health care system for SGTF using the TaqPath COVID-19 Combo Kit polymerase chain reaction assay (Thermo Fisher Scientific). We subsequently analyzed SGTF specimens for specific mutation target sequences found in Omicron and Delta variants using a polymerase chain reaction–based SARS-CoV-2 mutational panel (TaqMan SARS-CoV-2 Mutational Panel, Thermo Fisher Scientific) that had previously been validated for the detection of select mutations (ΔH69/V70, L452R, E484K, and N501Y) using well-characterized frozen archived clinical respiratory samples positive for Alpha, Beta, Gamma, and Delta variants (**Supplement**, available at Annals.org). To enhance Omicron detection, we incorporated 2 additional primer sequences (P681H and K417N) into the mutational panel and analyzed samples according to the following algorithm: Samples were analyzed using at least 3 mutation targets (L452R, K417N, and P681H); if the results of these target sequences resulted in an undetermined variant determination but a K417N mutation was identified, suggesting potential Omicron, the 3 mutation targets were repeated and 3 additional mutation targets were added (ΔH69/V70, L484K, and N501Y) to confirm the identification of Omicron. Variant determinations were made on the basis of the mutational profiles outlined in **Table 1**. To validate performance of this algorithm, we compared the results from the mutational panel to 119 clinical respiratory tract samples confirmed to be Omicron ($n = 69$) or Delta ($n = 50$) by WGS during this time frame (**Supplement**).

For the WGS-confirmed Omicron cases, we performed chart review of these persons, recording select clinical information (**Table 2**). This study was approved by the Mass General Brigham Institutional Review Board for the protection of human subjects under protocol 2019P003305.

From 1 December to 30 December 2021, we screened 2399 SARS-CoV-2 NAAT-positive specimens for SGTF. Of those, 1328 (55%) were positive for SGTF, with amplification of the ORF1ab and N gene targets. We identified 1260 of 1328 (95%) as Omicron and 14 of 1328 (1%) as Delta variants on the basis of mutation patterns listed in **Table 1**. In 54 of 1328 (4%) cases, a variant determination could not be made on the basis of the mutation profile; mean cycle threshold for these samples was 31.1 (SD, 4.9). Of the 69 samples that were confirmed to be Omicron by WGS, all were correctly identified by the mutational panel. Of the 50 samples confirmed to be Delta by WGS, 47 of 50 (94%) were correctly identified by the mutational panel; for the remaining 3 WGS-confirmed Delta samples, a variant determination could not be made on the basis of the mutation profile (**Supplement**). Sensitivity and specificity of the mutational panel for Omicron detection was 100%. Sensitivity and specificity for Delta detection was 94% and 100%, respectively.

Pertinent clinical characteristics of the 69 persons with WGS-confirmed Omicron infection are summarized in **Table 2**. Of note, 12 of 69 (17%) patients were referred for casirivimab–imdevimab or bamlanivimab–etesevimab therapy, with confirmed receipt in 8 patients (12%).

Discussion: Our results indicate that a real-time NAAT-based SARS-CoV-2 mutational panel accurately identifies mutations associated with Omicron, leading to correct identification of this variant among samples with SGTF. Although testing for SGTF was a useful screening tool for Omicron detection, the presence of SGTF alone did not predict identification of this variant in all cases and may be a less reliable surrogate for Omicron detection when prevalence is lower, underscoring the importance of more specific methods for real-time variant identification.

Table 1. SARS-CoV-2 Mutational Panel Mutation Targets and Expected Mutation Patterns for Omicron and Delta Variants and Mutation Panel Variant Determinations for 1328 SGTF SARS-CoV-2–Positive Respiratory Tract Specimens

Variant	ΔH69/V70	E484K	L452R	N501Y	P681H	K417N
Delta	− or UND* or ND	− or UND or ND	+	− or UND or ND	UND	− or UND
Omicron (BA.1)	+ or UND or ND	− or UND or ND	− or UND	+ or UND or ND	+	+
	+	− or UND or ND	− or UND	+ or UND or ND	UND	+
Undetermined	Mutational pattern that is not consistent with any of the above potential patterns					

	SARS-CoV-2 Mutational Panel Variant Determination	
	Sample Characteristics ($n = 1328$)	
	Percentage of specimens (n)	Mean Ct Value ± SD
Delta	1 (14)	28.2 ± 8.9
Omicron (BA.1)	95 (1260)	17.8 ± 5.7
Undetermined	4 (54)	31.1 ± 4.9

Ct = cycle threshold; ND = not done; SGTF = S gene target failure; UND = undetermined.
* A UND result occurs when there is low level or nonspecific amplification of a primer sequence due to low RNA content (Ct value ≥30), mixture of viruses that have both the reference or mutant sequences, or nonspecific amplification of an independent mutation underlying the mutation target of interest (e.g., P681H/P681R and E484K/E484A).

Table 2. Clinical Characteristics of Patients With Omicron Infection

Characteristic	Value
Total patients, *n*	69
Sex, *n* (%)	
Male	24 (35)
Female	45 (65)
Patient age, *y*	
Mean	38
Median	34
Range	2–88
Vaccination status, *n* (%)	
BNT162b2	32 (46)
BNT162b2 ×3 doses	12 (17)
BNT162b2 ×2 doses	19 (28)
BNT162b2 ×1 dose	1 (1)
mRNA-1273	19 (28)
mRNA-1273 ×3 doses	8 (12)
mRNA-1273 ×2 doses	11 (16)
JNJ-78436735	3 (4)
JNJ-78436735 ×1 dose	3 (4)
AZD1222	1 (1)
AZD1222 ×2 doses	1 (1)
Combined vaccination series	4 (6)
Combined series ×3 doses	3 (4)
mRNA-1273 ×2 doses, BNT162b2 ×1 dose	2 (3)
mRNA-1273 ×2 doses, JNJ-78436735 ×1 dose	1 (1)
Combined series ×2 doses	1 (1)
JNJ-78436735 ×1 dose, mRNA-1273 ×1 dose	1 (1)
Unvaccinated	6 (9)
Unknown vaccination status	4 (6)
Moderately or severely immune-compromised*, *n* (%)	5 (7)
Monoclonal antibody receipt†, *n* (%)	
Casirivimab–imdevimab	5 (7)
Bamlanivimab–etesevimab	2 (3)
Unknown formulation‡	1 (1)
Referred for therapy without confirmation of receipt	4 (6)
Total	12 (17)
Hospital admission, *n*	0

* Based on Centers for Disease Control and Prevention criteria for moderate to severe immunocompromise.
† Administration was based on Massachusetts General Hospital institutional criteria for monoclonal antibody allocation.
‡ Confirmed receipt of either casirivimab–imdevimab or bamlanivimab–etesevimab.

These results have important practical implications. Compared with the higher cost and slower turnaround time of WGS, the mutational panel provided less expensive, reliable results within the same day of testing using commercially available assays and instruments available in many clinical laboratories. Because casirivimab–imdevimab and bamlanivimab–etesevimab are ineffective against Omicron, these results would allow clinicians to make real-time determinations about appropriate allocation of monoclonal antibody therapy. Indeed, 17% of patients with confirmed Omicron infection were referred for either casirivimab-imdevimab or bamlanivimab-etesevimab, from which they were unlikely to benefit. Although sotrovimab has demonstrated retained activity against Omicron (1), supply of this therapy is limited, highlighting the importance of prompt Omicron identification to ensure optimal allocation.

Our study has some limitations. First, although we identified 1262 samples as Omicron using the mutational panel, we were only able to do confirmatory testing with WGS for 69 samples during this time period. Second, our study evaluated the accuracy of only the SARS-CoV-2 mutational panel among samples with SGTF; the ability of this panel to identify Omicron variants without using SGTF as a surrogate marker for ΔH69/V70 deletions (for example, BA.2) was not assessed. Third, the mutational panel evaluated in this study contains mutation targets that may have reduced performance for Omicron (ΔH69/V70 and P681H) because of nonspecific amplification of independent mutations similar to the mutation targets; we account for this potential limitation by including multiple mutation targets in the panel and using overall mutational patterns when making a variant determination. Finally, the mutational panel in this study does not include newer mutational sequences specifically designed for Omicron detection (G339D and Q493R); these targets should be evaluated for potential use in future mutational panels.

In conclusion, we show that a NAAT-based SARS-CoV-2 genotyping panel is an accurate and practical tool for real-time identification of Omicron; clinical use of these assays should be considered to help inform therapeutic decisions, particularly when effective therapy is in short supply.

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Reproducible Research Statement: *Study validation protocol:* See the **Supplement** (available at Annals.org). *Statistical code and genotyping data set:* Available on request from Dr. Turbett (e-mail, Turbett.Sarah@MGH.HARVARD.EDU). The code for genome assembly is available at https://dockstore.org/organizations/BroadInstitute/collections/pgs. All genomes have been submitted to NCBI GenBank; accession numbers are included in the supplement.

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